COMBINATORIAL IMPACT OF SNPS & microRNAs IN THE AETIOLOGY OF OVARIAN CANCER

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CERTIFICATE

This is to certify that the thesis entitled "**Combinatorial impact of SNPS & microRNAs in the aetiology of Ovarian Cancer**" submitted by **Ms. Seba Das** (Roll No. 413LS2028) in partial fulfillment of the requirements for the award of **Master of Science in Life Science** to the National Institute of Technology Rourkela is an authentic and original record of research work carried out by her under my supervision.

To the best of knowledge, the work incorporated in this thesis has not been submitted elsewhere for the award of any degree.

(Dr. Bibekanand Mallick)

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Rourkela

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Abstract

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation known to be associated with many cancer types including ovarian cancer, which is one of the leading causes of morbidity and mortality among women. Our present approach is focused on investigating the impact of SNPs residing within 3′UTR s of genes associated with ovarian cancer (OC) on microRNA (miRNA) targeting and alteration in target gene pool using our in house programming tool RNA hybrid. From this study we obtained two putative miRSNPs rs61764370_kRAS-hsa-miR-223-5p and rs2069414_CDK2-hsa-miR-183-5p having functional significance in ovarian tumorigeneseis. Further investigation and target validation will help us to achieve a deeper insight into unexplored variables of gene regulations linked to development and progression of OC.

Keywords: SNPs, miRNA, KRAS, CDK2, 3′UTRs

INTRODUCTION

INTRODUCTION

A disease in which number of the bodyôs cells after being malignant divide uncontrollably and spread into surrounding tissues is called as cancer. Cancer can begin virtually at any place within the human body. New cells are formed by cell growth and divided in order to fulfill bodyôs own requirement. Normal cells grow and upon being damaged, they die and are eventually replaced by new cells.

Cancer can be defined as a cluster of diseases causing abnormal cell growth having the potential of spreading to various parts of the body known as metastatic tumor or malignant tumor. All tumors are generally not cancerous like benign tumors, as they don't spread to different parts of the body and are found to be localized at specific location. Unlike most benign tumors present within the body, benign brain tumors may have life threatening properties. Potential signs and symptoms comprise of a replacement lump, abnormal hemorrhage, a chronic cough, weight loss. In addition, cancerous cells have ability to avoid signals that usually warn cells to prevent dividing or starts a procedure referred as apoptosis, in which our body removes unnecessary cells. Cancer cells also can be able to control the normal cells and circulatory system that give nutrition to a tumor cell. For example, cancer cells can induce nearby normal cells to create blood vessels that provide the tumors oxygen and nutrients for their growth. Blood vascular system additionally remove waste product from tumors. Cancer cells are typically ready to evade the organs, tissue, and particular cells of immune system that defend our body from disease and alternative situations. Our immune system generally removes abnormal cells from our body but some cancer cells are not detected by the immune system.

Cancer is caused by alteration of genes that control the mode of our cells performance, particularly the mode of growth and division. Characteristics of cancer cell include proliferation, cell division by activation of Ras or Myc oncogenes .Neoplasm suppression by Rb, inhibit growth and suppress and inactivate pathways that leads to modify cells to die. Tumor cells activate specific gene pathway that make them immortal even in one generations of growth. Cancer cell has a property known as angiogenesis that is the capability to get their own nutrition and blood vascular system and by metastasis they can spread inside body for getting nutrient from other healthy tissue.

Cancers are grouped in to 5 major types by the type of tissue in which the cancer originates.

(1) Carcinoma**:** cancers of epithelial tissue, e.g. the skin or lining of tissue or internal organs. Various types of carcinoma are Adenocarcinomas, Transitional cells, Squamous cell carcinoma, Basal cells carcinoma etc. (2) Sarcoma: cancer that originates from connective tissues such as bone, cartilage and tendon. These are less common type than that of carcinoma. The major types of sarcoma are soft tissue sarcoma, bone sarcoma, rhabdomyosarcoma etc. (3) Lymphoma: Cancer originates from cells of immune system such as lymphatic system where the immune cell starts diving abnormally in form of tumors. (4) Leukemia**:** cancer originates from blood forming cells such as bone marrow. In this type of cancer, the number of white blood cell increases in number. (5) CNS cancer: cancer of central nervous system i.e. tissue of brain and spinal cord.

There are over one hundred totally different known types of cancers diagnosed among humans such as hepatic cancer, pancreatic cancer, kidney cancer, cervical cancer, breast cancer, brain cancer and ovarian cancer etc. The most death causing cancers among females are breast, colon, endometrial, lung, cervical and **ovarian** cancer.

Ovarian cancer: is originate from cells of ovaries. Risk factors include infertility, medication for fertility, and increase body weight .The women have a family history of ovarian cancer have more chance of developing ovarian cancer. Ovarian cancer have hereditary effects. Hereditary ovarian cancer may have a less proportion of all cases of ovarian cancer.

Ovarian cancer is tough to detect at early stage because there are no specific symptoms. Detection include examination of pelvic region, X-ray tests, pelvic ultrasound and about one hundred twenty five test of the ovary. Most ovarian carcinoma growth in women are benign having fluid-filled cysts. The sterile women have high risk of ovarian cancer. Women have increase risk of ovarian cancer those start ovulation at a younger age or have menopause at a very older age. Hormone therapy after climacteric, fertility medication, and fleshiness increase risk of cancer.

Ladies with mutations in BRCA1 or BRCA2 have higher probability of developing this disease. Ovarian cancer is the most common form of cancer among women. Among 5 main subtypes of ovarian cancer, serous is commonest one. These tumors are believed to begin within the covering cell of the ovaries, most of cases it may form at the Fallopian tubes. Less common kinds of ovarian cancer include germ cell tumors and sex cord stromal tumors. A diagnosis of ovarian cancer is confirmed through examining histology of affected tissues.

Ovarian tumors starts from 3 common types of cell.

Epithelial ovarian cancer: Developing from the covering cells of the ovary. Most of the epithelial ovarian tumors are benign type. There are many categories of benign epithelial tumor, serous adenomas, mucinous adenomas and Brenner tumors. Cancerous animal tissue tumors are carcinomas - indicating they start within the lining of ovarian tissue. These are one of the most dangerous among different kinds of ovarian cancers. Sadly epithelial ovarian cancer is found in 70% of women and diagnosis is very less until the illness is at highly developed stage.

Ovarian germ cell tumor: The ova or eggs manufacture ovarian germ cell tumor. These cell have some properties of cancerous tumor and life threatening property .Generally germ cell tumors are non-cancerous in nature dysgerminomas, endodermal sinus tumors and maturing teratomas are the most common germ cell malignancies.

Ovarian stromal cancers: Ovarian stromal cancer developed from connective tissue. It starts from the cells that carry both the ovaries. Estrogen and progesterone are manufacture by them. Granulosa-theca tumors and Sertoli-Leydig cell tumors are most common types of stromal cancer.

Players behind Ovarian Oncogenesis:

Behind Ovarian cancer there are mainly 3 factors -

- Environmental factors (Advance age, Infertility, Endometriosis, Obesity, Family history), Genetic factors and epigenetic factors.
- Generally Genetic factors tends to alter the DNA sequence by mutation(deletion, duplication, insertion etc), ex-SNP

- Epigenetic factors donôt change the sequence of DNA .Ex- DNA methylation, histone modification and microRNA.

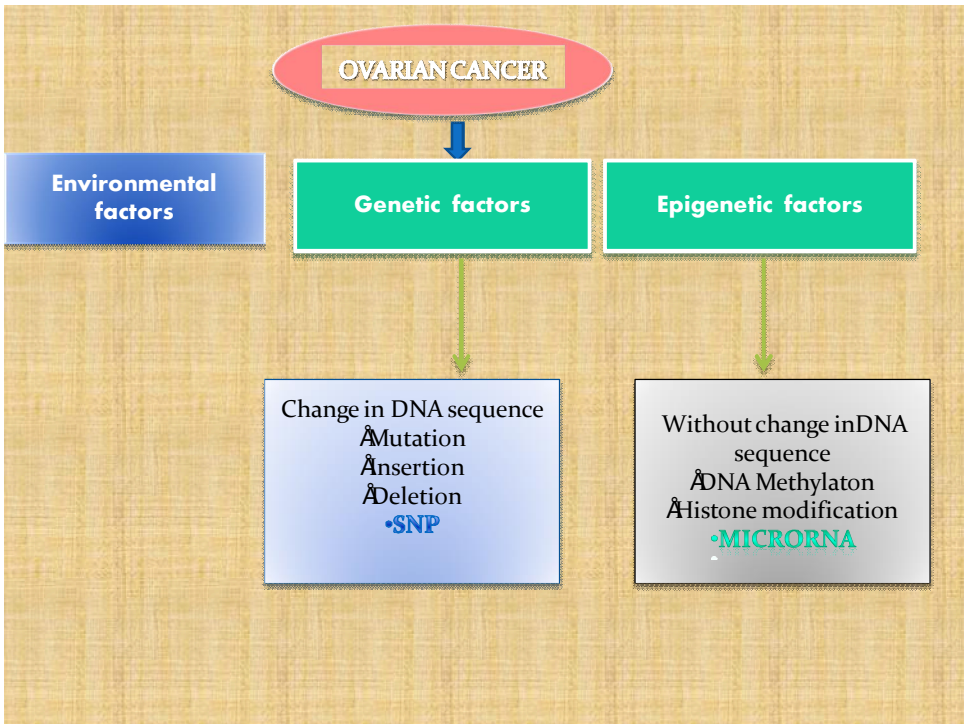

Figure-1- Causes of ovarian cancer

microRNA:

microRNA is known as small non-coding RNA, about 20-22 nucleotides length. In case of plants, animals, and some viruses have these types non coding RNA. RNA silencing machinery and post-transcriptional regulation of gene expression by degrading or silencing the target transcript is carried out ,by microRNA. Also in case of different biological processes like cell cycle, apoptosis, cell proliferation, carcinogenesis, metastasis and chemo resistance, microRNA plays an important role. In case of progression of cancers in human like ovarian cancer microRNA plays a vital role and it is proved that microRNAs act as tumor suppressor gene or oncogene. (Kinose et al., 2014)

SNPs

Single nucleotide polymorphisms, shortly named as SNPs. These are the most common type of genetic variation found in the genes. SNPs are population specific. SNP leads to change in a single DNA building block known as a nucleotide. Example, a SNP can substitute the nucleotide (G) with the nucleotide pyrimidine (A) at a certain stretch of DNA. A Single nucleotide change can have substitution, deletions or insertion effect in a nucleotide sequence. In case of coding region as well as in case of non-coding region of genes the SNPs are found. SNPs present in coding region of gene can alter the sequence of amino acid.

SNPs can be located in non coding region beside the CDS such as at 3ôUTR, 5ôUTR or in intron region. SNPs present in the UTR region are known to affect the functionality of target transcript, affecting the RNA interference mechanism operated by miRNAs. SNPs located within the 3 ÚTR are importance to unveil the possible role of these SNPs in disease pathogenesis as these are the hubs of miRNA binding and target gene regulations. SNPs located within 3/UTR can interfere with miRNA targeting either by creating or destroying the target site, thereby altering the expression of targeted genes. For instance, a SNP, rs4846049 (G>T) located within 3/UTR of 5,10-methylenetetrahydrofolate reductase (MTHFR) is identified to increase the risk of coronary heart disease through modifying miRNA binding for hsa-miR-149 (Wu et al., 2013).

Our present study is focused on investigating the impact of SNPs residing within 3/UTRs of genes associated with Ovarian cancer(OC) on miRNA targeting and change in target gene pool which will assist us to achieve a deeper insight for exploring variables of gene regulations linked to development and progression of OC. To achieve this goal, the aberrantly expressed up-regulated miRNAs and SNPs with 25 nucleotide flanking on each side of the polymorphic base reported in OC are used for predicting targets for both wild type and mutant alleles followed by identifying the potential miRSNPs by using stringent parameters of miRNA targeting such as seed topology, change in the hybridization energy, wobble base pairing within seed site, 3/ complementarity, 3/ supplementary sites and site accessibility that might be contributing to disease susceptibility and might give a clue towards tumor progression.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cancer is a class of diseases caused by abnormal cell growth. There are different types of cancer (about 100 types). When damaged cells divide uncontrollably to form lumps or masses of tissue termed as tumors, these enhance the starting of cancer. The digestive, nervous, and circulatory systems, excretory system are affected by the tumor and change the body function.A tumor is said as metastasized when it successfully spread to other parts of the body and grows, attacking and destroying other healthy tissues(Klein, 2008). This process itself titled as metastasis, and it is very tough to control. Cancer leads to uncontrollably growth without termination. Normal cells in the body follow a systematic pathway of growth, division, and finally death by apoptosis. Apoptosis is known as Programmed cell death and when cancer initiates to form this procedure breaks down. Like normal cells, cancer cells do not involved in programmatic death and it continue to grow and divide rapidly. This results to a mass of abnormal cells that grows and divides out of control.

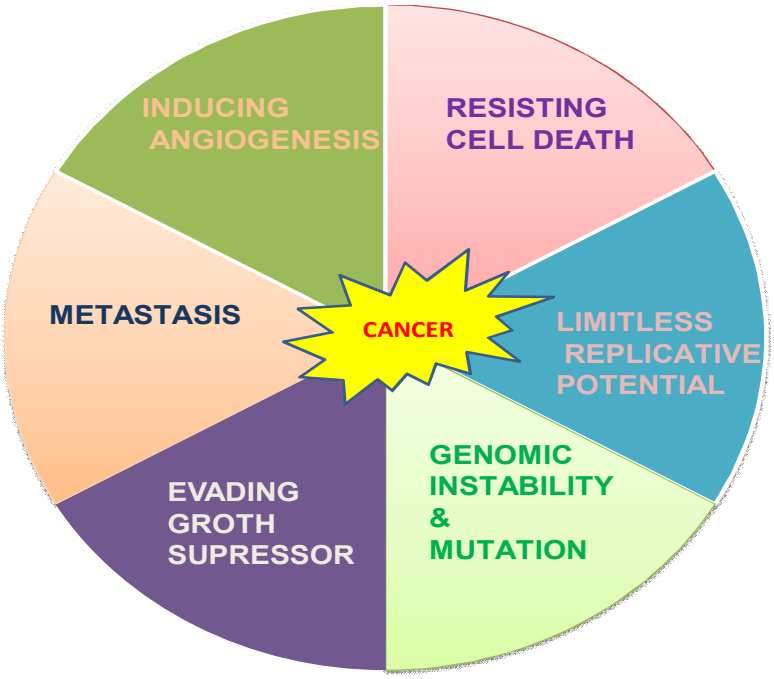

Figure 2- Characteristics of tumorigeneseis

Cancer is one type of complex group of diseases with many probable reason. There are various reasons of cancer like genetic factor and epigenetic factors which can be control by lifestyle of the individual like chewing of tobacco, unhealthy diet, various types of infection and exposure to different types of chemicals and radiation etc. There are some environmental factors including infertility, aging, tobacco, some viruses and bacteria, exposure to sun and radiation, different chemicals, certain hormones, alcohol, diet, lack of physical activity, family history of cancer, obesity, use of certain drugs etc.

Most occurring human cancer types are ï

Leukemia: Known as blood cancer. In leukemia the white blood cells are increase in number. But some leukemia found in other blood cell. It affects immunity system of our body. Different kinds of leukemia have different treatment.

Hepatic cancer: Also known as liver cancer. Liver cancer starts in cells of liver. abdominal pain, yellow skin, nausea and liver dysfunction are the basic symptoms of liver cancer.

Lung cancer: Lung cancer starts in cells of lungs. There are three main types of lung cancer. Small Cell Lung Cancer, Non-Small Cell Lung Cancer, and Lung Carcinoid Tumor.Most common type of lung cancer is Non-Small Cell Lung Cancer. Non-small cell lung cancers are about 85%. Subtypes of non-small cell lung cancer are Squamous cell carcinoma, large cell carcinoma, adeno-carcinoma. Small Cell Lung Cancer-Small cell lung cancer also titled as oat cell cancer. About 10%-15% of lung cancers are this type. This type of lung cancer spread quickly to other parts. Lung Carcinoid Tumor- 5% of lung cancers is lung carcinoid tumors. Also known as lung neuroendocrine tumors. The growth of this tumor is very slow and spread rarely.

Pancreatic cancer: It begins in the pancreas and most are of exocrine tumor types. Pancreatic neuroendocrine tumors, or islet cell tumors are less common type can have a better prognosis.

Breast cancer: Initiates from the tissue of breast. Lump in the breast, change in shape, fluid coming from the nipple, dimpling of the skin, and red scaly patch of skin are the symptoms of breast cancer.

Ovarian cancer: Ovarian cancer is the 5th most cancer among women. It has a most important role in morbidity and mortality with little variation in survival rates over past 30 years. Bloating, abdominal pain and discomfort, difficulty in eating, fatigue, indigestion, heartburn, constipation, early satiety, and possibly urinary symptoms are important symptoms of ovarian cancer. The etiology of ovarian cancer is not properly known.

Contributing factors of ovarian cancer are stimulation of extreme gonadotropin and androgen. Exposure of the ovaries to pelvic pollutants and carcinogens has an important role in the pathogenesis of ovarian cancer. Family history of disease plays an important factor of the disease. Mutations in the BRCA1 and BRCA2 genes responsible for ovarian cancer (Lancaster et al., 2015). Refractory infertility null parityare are the risk factors in ovarian cancer. Taking oral contraceptives and tubal ligation are the protecting factors. Use of oral contraceptive with 5 years continiously women may decrease their risk of ovarian cancer about in half (Holschneider and Berek, 2000).

Types of Ovarian Carcinoma

A wide variation of histological types of ovarian tumors is identified. Based on histogenetic principles the histological classification of ovarian tumors classified by the World Health Organization .These histological subtypes include epithelial ovarian tumors like endometrioid, serous, mucinous and clear cells.

The majority of malignant ovarian tumors are epithelial ovarian tumors, which further grouped into histological types such as serous, mucinous, endometrioid and clear cell. Clear cell and endometrioid carcinomas have a tendency to remain confined to ovary. In contrast Clear cell and endometrioid carcinomas are highly related with endometriosis. Clear cell and endometrioid carcinomas may be the unique histological types linked with serous carcinomas with respect to stage distribution and association with endometriosis (Kaku et al., 2003)

Signs and symptoms

There are various signs and symptoms of ovarian cancer .The signs and symptoms of ovarian cancer are frequently unseen in early stage. They may be indirect, even when they do at present. Before being known and diagnosed, symptoms will be present for several months in many cases. They may be primarily misdiagnosed as a condition like irritable bowel syndrome. The primary stages of ovarian cancer tend to be painless unless the growing mass results ovarian torsion.

Bloating, abdominal pain and discomfort, difficulty in eating, fatigue, indigestion, heartburn, constipation, early satiety, and possibly urinary symptoms are distinctive symptoms of ovarian cancer .These symptoms are initiated by a mass pressing on the other abdominopelvic organs. The presenting indications can include severe abdominal pain, irritation of the peritoneum, or hemorrhage in case of adolescents or children with ovarian tumors. Development and a growth of fluid in the abdomen start when the cancer becomes more advanced. Anything that raises a person's chance of developing cancer is called as a risk factor. Some risk factors affect the development of cancer directly and some indirectly cause cancer. Some people maynôt develop cancer with some risk factors, while others can develop cancer with no known cancer risk factors. Some known risk factors of ovarian cancer include-

Age

Age is an important factor of ovarian cancer. Women with increasing age have increasing risk of developing ovarian cancer. Women of all ages have a risk of ovarian cancer, women over fifty have more possible to develop ovarian cancer. Within the range of 32-55 age 68 % of women with ovarian cancer are found.

Family history

Women with family history have more chance to have disease .Women have 3 times higher risk of develop the disease with a first-degree relative (like mother, daughter, or sister) with ovarian cancer. When 2 or additional first-degree relatives are diagnosed with ovarian cancer the risk will increase.

Genetic factors

Because of genetic mutation about 10% -15% of ovarian cancers happen. There are several genetic condition related to an increased risk of ovarian cancer. The women with an epithelial ovarian cancer have become readily apparent with identification of the BRCA1, and BRCA2 genes. Women have a high risk of ovarian cancer, who had mutation in either of these genes (11ï 60%). These are the highly lethal cancers. In families with a strong history of ovarian cancer, BRCA1and BRCA2 mutation testing has become the accepted standard of care. (Gau et al., 2015). Mutations can alter functioning of gene via change in the nucleotide sequence either by insertion or deletion or Single nucleotide polymorphism (SNP).

Epigenetic factors

Epigenetic factors donôt change the sequence of DNA, occur in various cancers affect transcription of various genes act by DNA and Histone modification or ncRNA (miRNA) mediated target repression. The transcriptional regulation of miRNAs in association with SNP remains an unexplored area in many cancer types including ovarian cancer.

Single nucleotide polymorphisms (SNP)

Single nucleotide polymorphisms (SNPs) are common type of genetic variation known to be associated with many diseases. Each SNP cause a single change in a DNA building block (Nucleotide). For instance- a SNP may replace the nucleotide thymine (T) with the nucleotide cytosine (C) in a certain stretch of DNA. Throughout DNA SNPs are present. once in every 300 nucleotides on average the SNPs are found, There are approximately 10 million SNPs in the human genome (Shastry, 2002). Generally in the DNA level between genes these variations are found. They can act as biological markers, helping scientists to find genes with genetic variation that are associated with any abnormal condition like cancer. They may play a direct role in disease by affecting the gene When SNPs present within a gene or in a regulatory region.

(Shastry, 2009)

Figure-3. Single nucleotide polymorphism

Polymorphism at the DNA level has been playing an increasing factor in animal genetic studies. Amongst others, the microsatellite DNA marker has been the most marker type, named SNP, for Single Nucleotide Polymorphism, is now on the scene and has gained high recognition, even though it is only a bi-allelic type of marker (Vignal et al., 2002). DNA sequence is formed by a chain of 4 nucleotide bases: A, T, C and G. The variation can be categorized as SNP if more than 1% of a population does not have the same nucleotide at a specific location in the DNA sequence. The gene is defined as having more than one allele if a SNP present within a gene. Variations in the amino acid sequence caused by SNPs. SNPs are also present in noncoding regions of DNA (Ramensky et al., 2002). A specific SNP may not responsible for a disorder but some SNPs are associated with certain diseases. Scientists may study stretches of DNA near SNPs in an attempt to classify the gene or genes responsible for the trait if certain SNPs are recognized to be associated with a trait.

Despite this, a Single nucleotide polymorphism can present in two regions known as-

1. Coding region 2. Non region

Changes in translational product takes place if a SNP lies in the coding region of a gene i.e. protein formed. This phenomenon can again be by two types- non-synonymous and synonymous. Synonymous region donôt affect the protein sequence while non synonymous region cause changes in the change amino acid sequence ultimately affecting the protein conformation (Cargill et al., 1999). Again non-synonymous change can occur in missense codon and nonsense

codon. In missense codon change results in different amino acid and non-sense codon are known as premature stop codon.

In the case where mutation (SNPs) lies in noncoding regions, gene expressions are likely to get affected in other way. Generally non-coding regions such as 5ðUTR, 3ðUTR, introns, intergenic Regions are known to be associated with gene splicing mechanism , regulatory miRNA binding mechanism, mRNA degradation processes etc. all together contributing to regulation of target gene expression.

Because of the population specific occurrence of SNPs, these are generally used to find the genetic similarity among a population. The genetic variation caused by the presence of SNP can be implicated in finding the disease causing gene that can be further used as a disease biomarker. The variability in drug response among different population can be studied on the basis of presence of specific SNPs.

SNPs AND CANCER

Genetic variation in human genome is emerging resource for finding out cancer, a complex set of diseases defined by each genetic and environmental factor. The common germ-line variantôs quality is great, on the order of 10 million per person and shows a remarkable chance to analyze the etiology, interindividual variations in treatment response and outcomes of specific cancers types. The critical determinants in environmental exposure will clarify by study of genetic variation and cancer that may have future implications for preventive and early intervention procedures. (Erichsen and Chanock, 2004).

SNPs AND OVARIAN CANCER

There are sample of studies like Genome wide association studies screening the role of SNPs in the occurrence of ovarian cancer. One such study indicates the risk of gynecological cancer and the association of SNP rs763110 . The results suggested that the FASL polymorphism may decrease the risk of gynecological cancer (Zhou et al., 2015). Another report shows single-nucleotide polymorphisms (SNPs) present on chromosome at position 5p15results TERT and CLPTM1L mutation and significant role in endometrial cancer (Carvajal-Carmona et al., 2015).

The rs231775 allele present in the coding region association results in change of amino acid in CTLA4 finally contributing to ovarian cancer. SNPs seem to play a vital role in ovarian cancer survival, mainly in patients with endometrioid epithelial ovarian cancer and clear cell has been reported sometimes acting as biomarker of ovarian Oncogenesis (Charbonneau et al., 2014). The union of genetic variants in the FGF (fibroblast growth factor) pathway in therapeutic reaction of ovarian cancer has been elucidated with the discovery of SNPs through FGF-FGFR pathway (Meng et al., 2014). This provides a molecular approach for monitoring therapeutic response,and prediction of ovarian cancer diagnosis.

SNP regulates the miRNA targeting which additionally one of the causes of various cancers including Ovarian cancer types. miRNAs are known to bind 3ô UTR or 5ôUTR of target mRNA regulating its functioning and finally contributing to different cellular activities like growth control, differentiation as well as human disease like cancer (Saunders et al., 2007). SNP affects the miRNAs binding when in a miRNA target site thus altering the miRNA mediated regulation machinery. According to the current annotation study of miRNA gene and SNP provide the detail of modification of miRNA targeting done by SNP. Present of SNP in seed region can change the miRNA-mRNA targeting either by creation or destruction of binding site thus affecting the protein translation mechanism (Mallick and Ghosh, 2011).

microRNA

MicroRNAs are newly discovered class of non-coding RNAs that have key roles in the gene expression regulation. The miRNAs are small 20-22 nucleotide long single stranded noncoding RNA which takes part in posttranscriptional gene silencing process. miRNAs play major role in different biological events such as differentiation, organogenesis, apoptosis ,cell proliferation and tumorigeneseis process (Hwang and Mendell, 2007). The aberrant expression and deregulation of miRNA making it unable to bind with 3ôUTR of the target mRNAs are concerned in risk of various diseases such as tumorigeneseis. miRNAs target more than 30% human genes (Lewis et al., 2005).

miRNA are mainly encoded in intergenic regions or protein coding genes . Intergenic miRNA transcription of from primary miRNA takes place by the catalyst activity of RNA polymerase II and RNA polymerase III (Morlando et al., 2008). miRNA present at intron are co-transcribed with their host genes (Wang et al., 2009). Many proteins and enzymes are involved in biogenesis of miRNA such as- two enzymes known as Drosha and its subunit DGCR8 who participate in cleavage of pri-miRNA transcript and generate a hairpin precursor within the nucleus, known as pre-miRNA (Han et al., 2004). Hairpin stem pri-miRNA contain thirty three base pairs with terminal loop and upstream of the hairpin and also at downstream. Drosha slice eleven nucleotide away from hairpin base , known as a identification part for DGCR8 binding that determines Drosha cleavage site . The pri-miRNA loops additionally act as a binding site for nuclear ribonucleoprotein A1 sometime. After finishing the action of Drosha, pre-miRNA is then exported from nucleus to the cytoplasm by a nuclear transport receptor complex known as expotin-5-RanGTP. For formation multiple category of small non-coding RNA dicer acts as catalysis. Dicer is aided by trans- activation responsive RNA-binding protein additionally known as TRBP within the mammals system. Inside the cytoplasm dicer with few accessory protein processed this pre-miRNA into mature 22 nt miRNA and the method of miRNA biogenesis is completed. Then in next step pre-miRNA with Argonaut protein for mRNA targeting forms the RNA inducing silencing complex (RISC) (Murchison and Hannon, 2004). Throughout the loading process ,Argonaut protein cleaved non-guided strand. Ago protein take part in gene silencing pathway and among various species it is conserved.

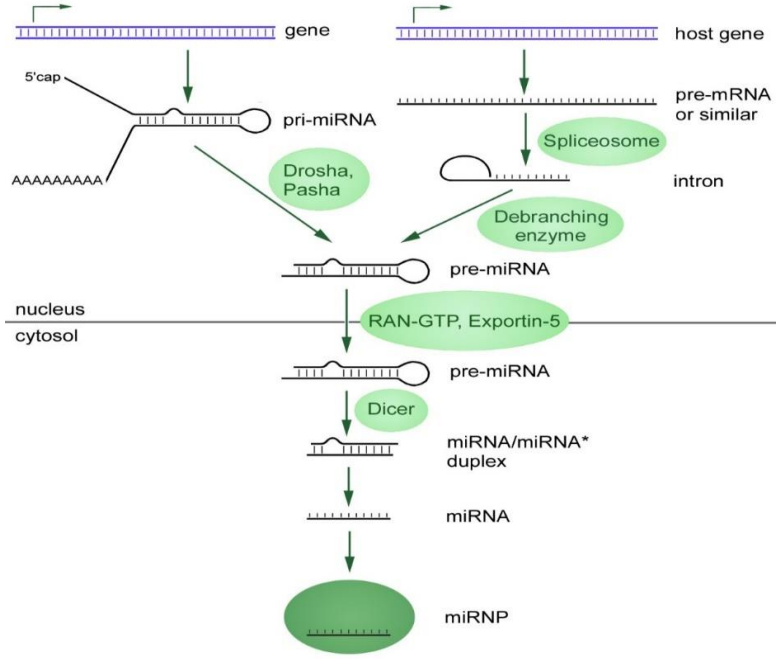

Figure-4. miRNA biogenesis pathway(Adopted from en.wikipedia)

Mature miRNA target the mRNA and direct mRNA translational repression and destabilization. 2-6 nucleotides of miRNA that binds with mRNA is known as the Seed region explained by base pairing rule of Watson and crick (Brodersen and Voinnet, 2009). For miRNA target recognition the Seed region is the most important site. Some basic characteristics as well as requirement of miRNA binding are- (i) presence of binding position that should away from the center of UTR region, (ii) occurrence of A-U rich nucleotide composition near the target region, (iii) feasibility of mRNA UTR regions making it available for miRNA binding (iv) presence of multiple site for a specific miRNA known to have greater effect in regulation.

There are basically 3 types of target site such as- canonical site, marginal site, and atypical site. The canonical sites can be again categorized as- (a) 7mer-A1 site : designated with 2-7 nucleotides from the 5ô end ,along with presence Adenine(A) is in target mRNA at 1st position of miRNA binding, (b) 7mer-M8 : with binding position from 2 to 8 in miRNA and (c) 8mer : having 2-8 base pairing with the presence Adenine(A) is on the target mRNA corresponding to first 1st position of miRNA. Likewise marginal site can be of two types-(i) 6mer: with 2-7 base pairing and (ii) offset 6mer: having 3-8 base pairing with the target transcript. The atypical site can also be categorized in to two such as 3ôSupplementary site and 3ôcompensatory pairing that provide additional pairing support to the miRNA binding (Friedman et al., 2009). Different types of investigational confirmation has proved that 3ôsupplementary pairing in increasing affinity of seed pairing and the binding specificity. Whereas in case of compensatory site the Watson-crick pairing usually centering on miRNA nucleotide 12-16 can balance for seed mismatch and thereby make a functional site for binding. In a seed region miRNA has many binding sites but 8mer binding site is the most efficient bindind site (Bartel, 2009).

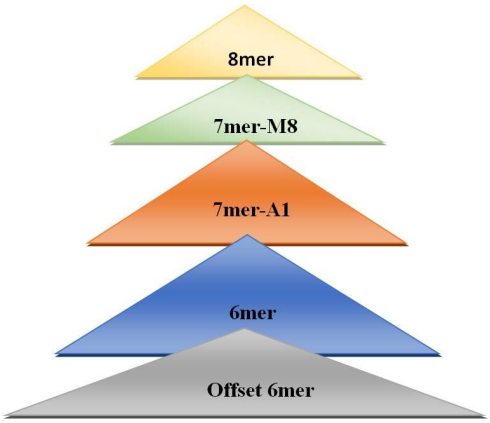

Figure-5. Binding efficiency of target sites shown in increasing order from top to bottom

Our present study aimed on investigating the impact of SNPs residing within 3/UTRs of genes associated with ovarian cancer (OC) on miRNA targeting and alteration in target gene pool which will help us to achieve a deeper insight into unexplored variables of gene regulations linked to development and progression of OC. To achieve this goal, the aberrantly expressed up-regulated miRNAs and SNPs with 25 nts flanking on each side of the polymorphic base reported in OC are used for predicting targets for both wild type and mutant alleles followed by

identifying the potential miRSNPs by using stringent parameters of miRNA targeting such as seed topology, change in the hybridization energy, wobble base pairing within seed site, $3^{/}$ complementarity, $3^{/}$ supplementary sites, site accessibility that are that might be contributing to disease susceptibility and might give a clue towards tumor progression.

OBJECTIVES

OBJECTIVE 1: DATA RETRIEVAL

(a) Collection of ovarian cancer specific SNPs

(b) Retrieval of Differentially expressed miRNAs in ovarian cancer

OBJECTIVE 2- RNA Hybrid study in order to find possible miRSNPs

OBJECTIVE 3- Identification of significant miRSNP pairs

OBJECTIVE 4- Functional annotation of selected pair of miRSNPs

MATERALS AND METHODS

Materials and methods

1. Data retrieval: Collection of SNP

Data retrival from literature studies carried out to find those differtially expressed single nucleotide polymorphisms(SNPs) reported by different GWAS studies associated with ovarian cancer at different population. SNPs obtained were found to be present in different genic regions such as exon, 3ðUTR, 5ðUTR, intron, CDS. The sequence information of the SNPs were retrieved from dbSNP database, literature and SNPedia. Then the SNPs present in 3ðUTR region which is known to be the potential binding site of miRNAs were consider for further validation.

2. Expression data mining:

2.1. Gene Expression Data:

Genome wide analysis of miRNAs from normal and diseased sample in ovarian cancer was done in order to differentiate between different expression pattern for diagnosis and therapeutic mechanisms from GEO (Gene Expression Omnibus) database.

Gene Expression Omnibus is a data base from where the expression data can be retrieved resulting from different experimental procedure such as microarray, small RNA sequencing etc. A collection of web based interfaces and applications are available for downloading gene expression data for different disease from GEO database.

The miRNA expression data of the ovarian cancer and corresponding normal control sample was taken for analysis.

2.2. Microarray analysis of gene Expression Data:

All samples of ovarian cancer (37 endometriod, 41 serous, 13 mucinous, 8 clear cell) and normal control (i.e. all GSMs) present in the GSE were taken for analysis. The basic steps include:

- Data were taken from GEO dataset in multiples to decrease error.
- Raw data files were downloaded in .CEL format.

- The files were kept in unzipped folder, extracted and renamed as test and control for both mRNA and miRNA .

3. Analysis of Gene Expression Data:

For miRNA expression analysis Agilentôs GeneSpring GX 12.6 software was used. This is a microarray expression data analysis tool for analysis and fast visualization of different microarrays (genome copy number ,gene, miRNA, exon etc). This tool is designed for satisfying the requirements of biologists. Performing work in GeneSpring GX 12.6 is organized into projects. Number of experiments are involved in a project and an experiment is composed of samples (data sources). Samples are grouped as control and test. Interpretations were done based on the defined parameters of sample grouping and finally analysis involves a number of statistical steps and corresponding results.

In Genespring GX all information about microarray design and biological information is found. Initially for each new array type which is to be analyzed, a new technology must be installed. Technologies created include affymetrix for standard arrays, Agilent and Illumina. In Gene Spring analysis there are following steps-

(a)Gene expression analysis:

In order to minimize the systematic non-biological differences and to reveal true biological differences, normalization of data was done, after which profile plot of normalized intensity map values are obtained. To standardize each chip for cross comparison, data is normalized to 75 percentile of signals intensity. The main purpose is to eliminate redundancy and make sure that the data makes sense with minimum number of entities.

For analysis of gene expression a new experiment was created. The experiment type should be specified as Affymetrix. Workflow selected was Guided Workflow. Using choose sample option is the unzipped samples were uploaded to the experiment from the saved location in the system. Experimental grouping was done to define samples as test and control and assigning a parameter name (e.g. Average). By using Filter Probsets by Errors, quality control of samples was done. This was done on the raw signal values of all the entities. For filtering, cut off was set at 20

percentile of all intensity values and a profile plot of filtered entities was generated. Using the normalized signal values and grouped samples by active interpretation box whisker plot is generated.

Significance analysis depending upon experiment grouping, was done by performing T-Test Unpaired analysis as 2 groups are there that is control and test along with replicates. For computing p-values Benjamin-Hochberg FDR algorithm multiple testing correction was used. The p-value cut off taken was Ò0.05. This reduces the number of false positives or false discovery rate. This multiple testing correction is least stringent. Fewer chances of false negative genes are there. A p-value of Ò0.05 is taken as significant. For identifying differentially genes among the cancerous and non-cancerous samples which are expressed above a definite threshold, fold change analysis is done. It gives the absolute ratio of normalized intensities between the average intensities of grouped sample. Fold change cut off is taken Ó2.0. Further 2D hierarchical clustering of the genes expressed >2.0 fold was carried out taking average linkage to classify the cancerous and normal control samples and a heat map was generated using CLUSTER 3.0 and JAVA tree view. Hierarchical clustering method arranges gene in a tree structure based on their similarity. If the items are similar to each other then they are connected by short branches and if it is dissimilar then it is connected by long braches. Genes expressed greater than 2 fold were exported from Gene Spring along with normalized signal values, gene symbols, entrez gene IDs etc. The entity list was exported as .txt file and later opened with excel for further analysis.

(b)For miRNA expression analysis:

miRNA expression analysis was carried out in the same project of GeneSpring, but in a new experiment. miRNA specific platform i.e. Illumina was chosen and further steps of analysis were same as that of mRNA expression analysis. The miRNAs differentially expressed greater than 2 fold between the normal and cancerous samples were exported for further analysis.

4. RNA hybrid Analysis

Differentially expressed up-regulated miRNAs and SNP sequences with 25 nts flanking on each side of the polymorphic base are used for predicting targets using our in-house multi-step

predictions tool RNAhybrid for both wild type and mutant set of sequences **.**It is a tool for finding the minimum free energy of hybridization of a long and a short RNA.

5. Filtering out in order to find the most probable miRSNP pair

The RNAhybrid list was filtered on the basis of following parameters:

Change of binding site:

Seed region is the most important factor for miRNA target anticipating a perfect or near perfect binding between the mature miRNA and its target. Change in binding site between the ancestral and mutated were designated as: Site Creation (mer to other) or Site destruction (other to mer). The miRSNP pairs were filtered out by above parameter with most appropriate binding site.

Variation in Seed topology:

Seed topology indicates the type of seed site present in the miRNAs. Due to the presence of SNP in these seed site various new seed types are supposed to be formed different from the ancestral one that can vary with many seed topology like 7mer, 8mer, 7merA1, offset 6mer, 6mer etc. Change in seed types with most potential seed topology were filtered from the existing list.

MFE change:

This is otherwise known as minimum free energy change resulting due to the miRNA binding to the target mRNA. We set the parameter of MFE change that should not be less than -10. Because inside cell miRNA targeting is done with high minimum free energy. A reasonable threshold for MFE is required because it is one of the important parameter that one can set to increase the stringency of the miRNA target duplex mimicking to the cellular context.

Presence GU in the seed site:

Presence of GU indicates unusual pair that differs from the normal Watson crick base pairing. It donôt obey Watson crick principle. So presence of GU should be less (0 or 1) because occurrence of more GU in the seed site can interfere with miRNA binding and base pairing.

Presence of Additional binding sites beyond seed site:

Presence of supplementary binding or compensatory binding sites e.g. in 12-17 beyond the seed site is known to strengthen binding of miR to the target.

The most suitable miRSNP pairs were retrieved those who satisfied the above all parameters.

6. Establishing 1 Gene - 1 miRNA Hypothesis

This hypothesis was experimented by the help of database Targetscan in order to establish the fact that mRNA present in the pair is targeted by only one conserved miRNA which is also present in the pair. Target Scan predicts biological targets of miRNAs by searching for the presence of conserved sites that match each miRNA (Lewis et al., 2005). As an option, nonconserved or poorly conserved sites are also predicted. Also identified are sites with mismatches in the seed region that are compensated by conserved 3' pairing (Friedman et al., 2009). In mammals, predictions are ranked based on the predicted efficacy of targeting as calculated using the context+ scores of the sites. Here we searched the target of conserved sequence of miRNA with our gene of interest. Target Scan provides accurate rankings of the predicted targets for each miRNA.

6. Searching for presence of other binding site for the miRNA in the target beyond miRSNP binding site

- RNAhybrid is a tool for finding the probable miRNA-mRNA binding site with appropriate parameters.
- In order to find other binding sites of miRNA, first we retrieve the sequence of mature microRNA from miRbase, a database of published miRNA sequences and annotation.
- Then we collected 3 ôUTR sequence of gene of interest from NCBI in FASTA format.

- Then both the sequence were uploaded in prescribe format of RNAhybrid-BiBiserver with parameters like Helix constrent-2-7, MFEð 10.

8. Functional annotation through Gene Ontology Analysis

The functional significance of selected miRSNPs were retrieved by checking through individual associated gene function and expression in ovarian the disease system using **Metacore**.

Metacore is an integrated software suite for functional analysis of Next Generation Sequencing, variant, CNV, microarray, metabolic, SAGE, proteomics, siRNA, microRNA, and screening data. This is an advanced database for path way analysis. Metacore is based on a high-quality, manually-curated database of: Transcription factors, receptors, ligands, kinases, drugs, and endogenous metabolites as well as other molecular classes. Rich ontologies for diseases and processes with hierarchical or graphic output. By the help of Metacore we got various function and regulation of our gene of interest present in the miRSNP in ovarian cancer system.

Additionally extensive literature search was done separately for the functional significance of the pair in various other systems including ovarian cancer.

The expression pattern of the genes were collected from **Oncomine,** a cancer-profiling database containing published data that has been collected, standardized, annotated and analyzed by Compendia Bioscience. Oncomine currently includes gene expression and sample data from 500 cancer types and a wide range of cancer-related cell lines. There are more than 490 datasets and nearly 40,000 measured samples, including cell line panels representing published datasets and microarray experiments, respectively. Experimental details and sample facts are collected from supplemental data and direct correspondence with authors of published work and added to the database to drive new analysis. The data are normalized and analyzed using standard protocols, and presented to the end-user of Oncomine through a web-based interface. From this database we got information about our gene of interest in ovarian cancer cell line.

RESULTS AND DISCUSSIONS

Results & Discussions

1. List of SNP present in 3'UTR

132 SNP associated with ovarian cancer retrieved from literature, dbSNP, SNPedia. From 132 snp only 22 SNP are present in 3ÚTR location. (Table1)

SNP	Allele	GENE	Location	Source
rs3733336	G/A	FGF5	3' UTR	PMID: 24146310
rs3781699	A/C	LRRC32	3' UTR	PMID: 23894717
rs1052587	C/T	MAPT	3' UTR	PMID: 23535648
rs17574361	A/G	KANSL1	3' UTR	PMID: 23535648
rs4640231	C/G	CRHR1	3'UTR	PMID: 23535648
rs16917496	C/T	SET8	3' UTR	PMID: 22867998
rs61764370	T/G	KRAS	3' UTR	PMID: 20647319
rs1042364	G/A	ADH4	3' UTR	PMID: 21480392
rs6990375	G/A	SULF1	3' UTR	PMID: 21214932
rs13420827	G/C	DNMT3A	3' UTR	PMID: 18381459
rs5275	C/T	PTGS2	3' UTR	PMID: 20559705
rs608995	T/A	PGR	3'UTR	PMID: 20547493
rs10131	G/A	LIG4	3' UTR	PMID: 20386703
rs2278414	G/A	ZBRK/ZNF350	3' UTR	PMID: 20306497
rs11169571	T/C	ATF1	3' UTR	PMID: 19950226
rs1425486	T/C	PGDFC	3' UTR	PMID: 21118967
rs2069414	C/A	CDK2	3' UTR	PMID: 19258477
rs1053578	T/C	ACBD4	3' UTR	PMID: 20111712
rs1053578	T/C	ACBD4	3'UTR	PMID: 20111712
rs1425486	T/C	PDGFC	3'UTR	PMID: 21118967
rs2069414	C/A	CDK2	3'UTR	PMID: 19258477
rs608995	T/A	PGR	3'UTR	PMID: 15632380

Table 1- List of SNPs In 3âutr

2. Microarray Analysis

Agilentôs GeneSpring GX 12.6 software was used for gene expression analysis. This software is a powerful microarray expression data analysis tool for fast visualization and analysis of miRNA as well as mRNA Microarrays. Class of transcripts showing expression pattern which are correlated with experiment variables are identified by using this software. The expression analysis of differentially expressed miRNAs were retrieved and is shown in following clustering diagram.

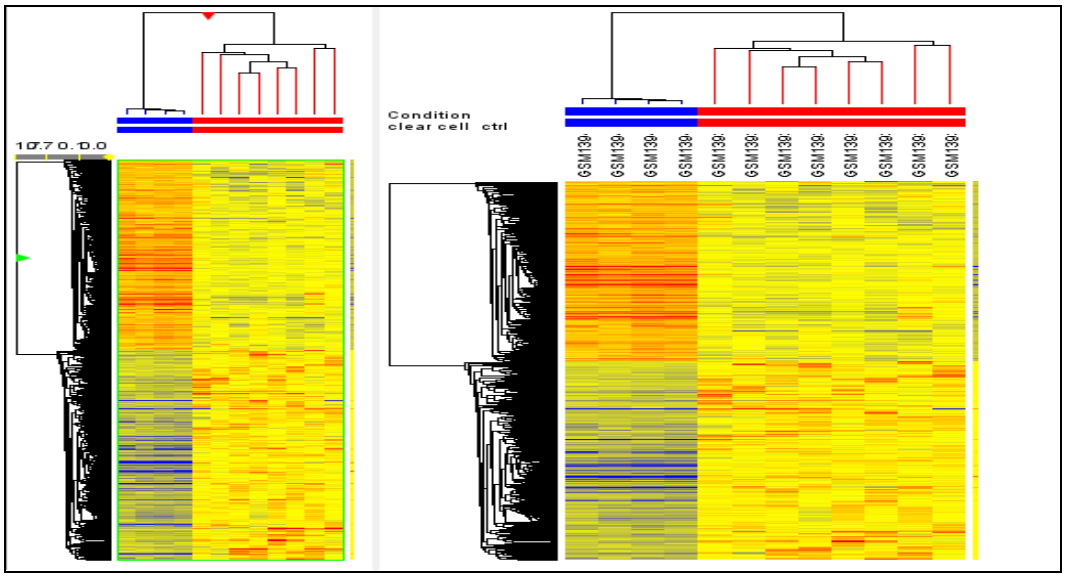

Figure-6 . Clustered analysis showing DE mRNAs in Clear cell ovarian carcinoma

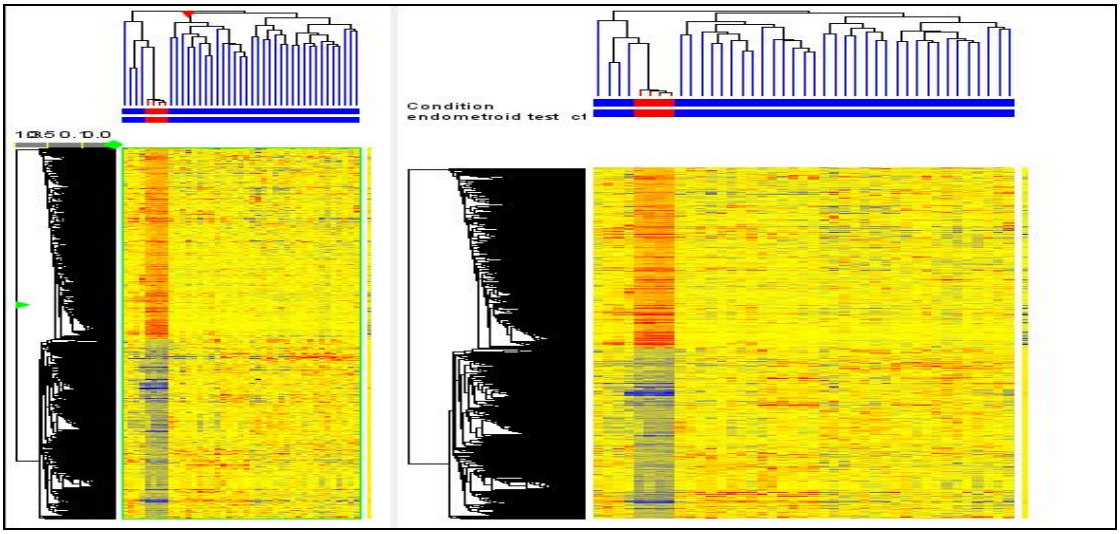

Figure-7. Clustered analysis showing DE mRNAs in Endometrioid ovarian carcinoma

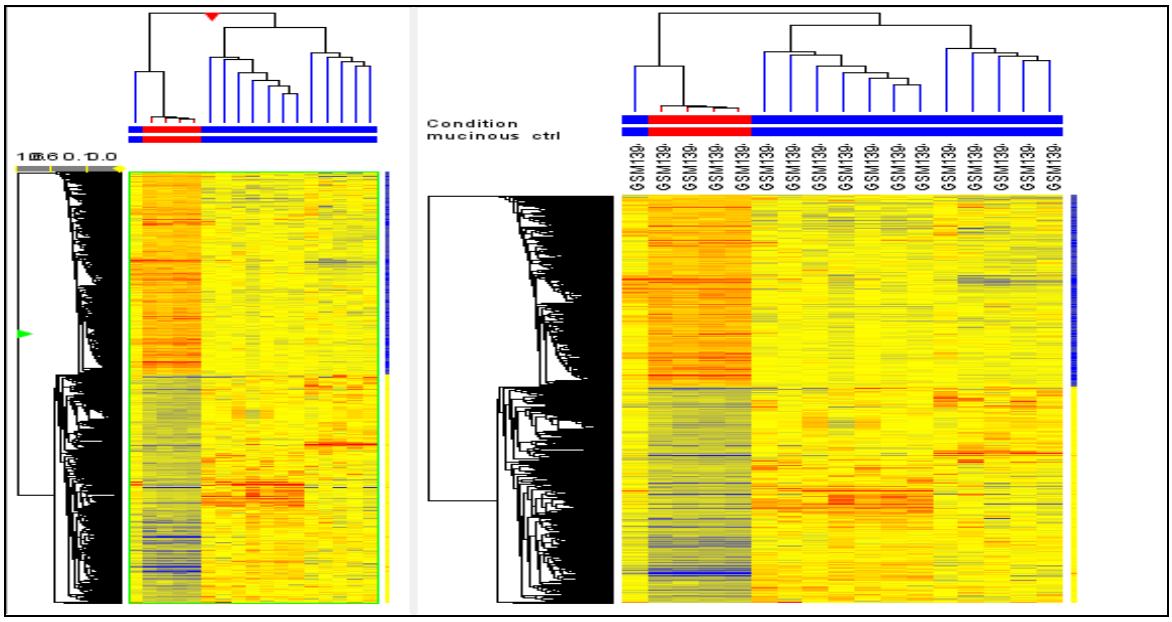

Figure-8. Clustered analysis showing DE mRNAs in Mucinous ovarian carcinoma

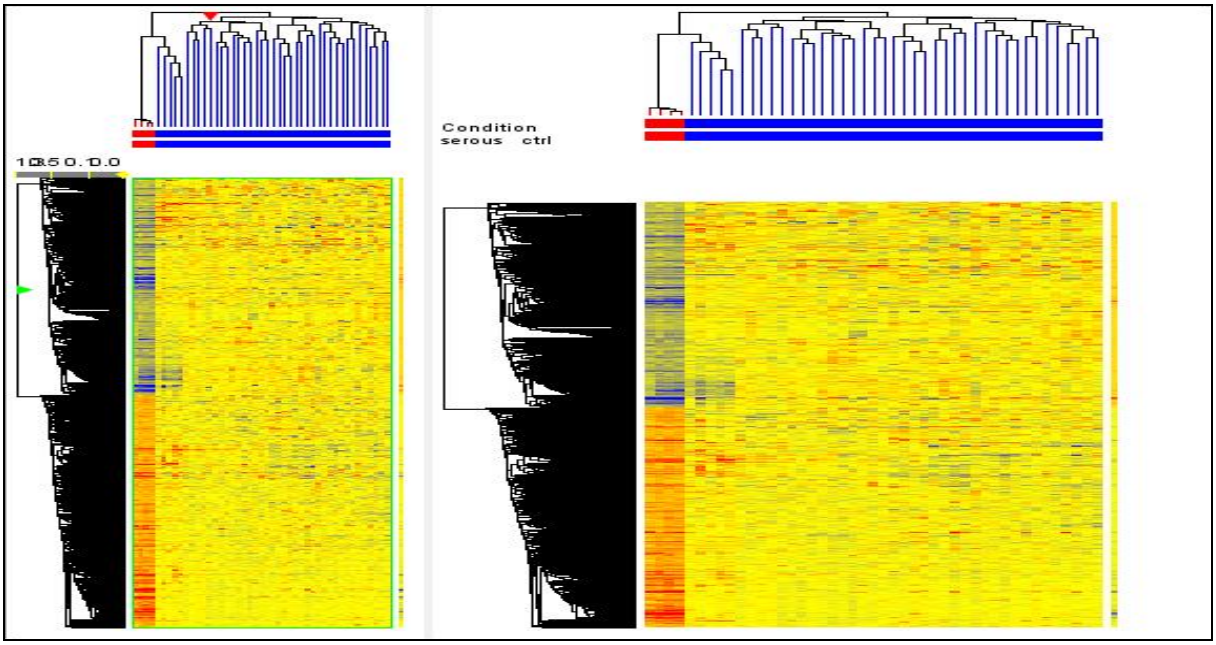

Figure-9. Clustered analysis showing DE mRNAs in serous ovarian carcinoma

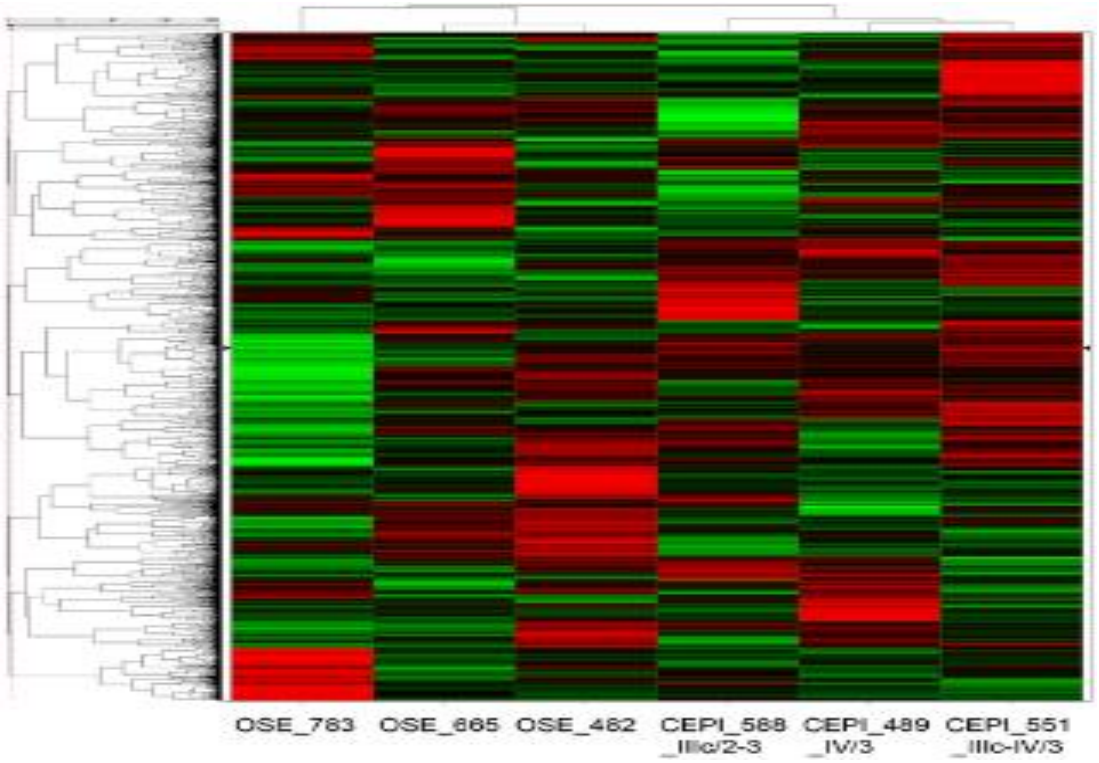

Figure-10. Clustered analysis showing DE miRNAs in ovarian carcinoma

3. Result of RNAhybrid

We took 23 SNP (3ðUTR) AND 149 up regulated miRNA express in ovarian cancer for RNAhybrid analysis. From RNAhybrid analysis we got 137 pair of miRNA-SNP. RNAhybrid was run to study interaction among the chosen up regulated miRNA and 3ðUTR SNP pair.

4. Filtered most Probable miRSNPs

From 137 pair obtained from the RNA Hybrid, 46 miRSNPs were filtered on the basis of different parameters like- change of binding site, variation in Seed topology, MFE change, presence GU in the seed site and Presence of Additional binding sites beyond seed site. Among **46** pair of miRSNP, 27 pair were with new site creation and 19 sites with destruction of sites as compared to the ancestor allele.

SNP-gene	miRNA	Change in seed type	Seed Type
rs1053578_ACBD4	hsa-miR-28-5p	Site creation	Other-7mer-m8
rs11169571_ATF1	hsa-miR-340-5p	Site creation	Other-7mer-m8
rs13420827_DNMT3A	hsa-miR-155-5p	Site creation	Other-7mer-m8
rs1052587_MAPT	hsa-miR-199a-5p	Site creation	Other-6mer
rs5275_PTGS2	hsa-miR-129-5p	Site creation	Other-6mer
rs2069414_CDK2	hsa-miR-206	Site destruction	6mer-other
rs1425486_PGDFC	hsa-miR-218-5p	Site destruction	7mer-other
rs61764370_KRAS	hsa-miR-223-5p	Site destruction	6mer-other
rs1052587_MAPT	hsa-miR-133a-5p	Site destruction	7mer-A1-other
rs2069414_CDK2	hsa-miR-183-5p	Site destruction	7mer-A1-other
rs2069414_CDK2	hsa-miR-1	Site destruction	6mer-other

Table 2 significant miRSNP pair

5. Expression pattern of genes in selected miRSNPs from Oncomine

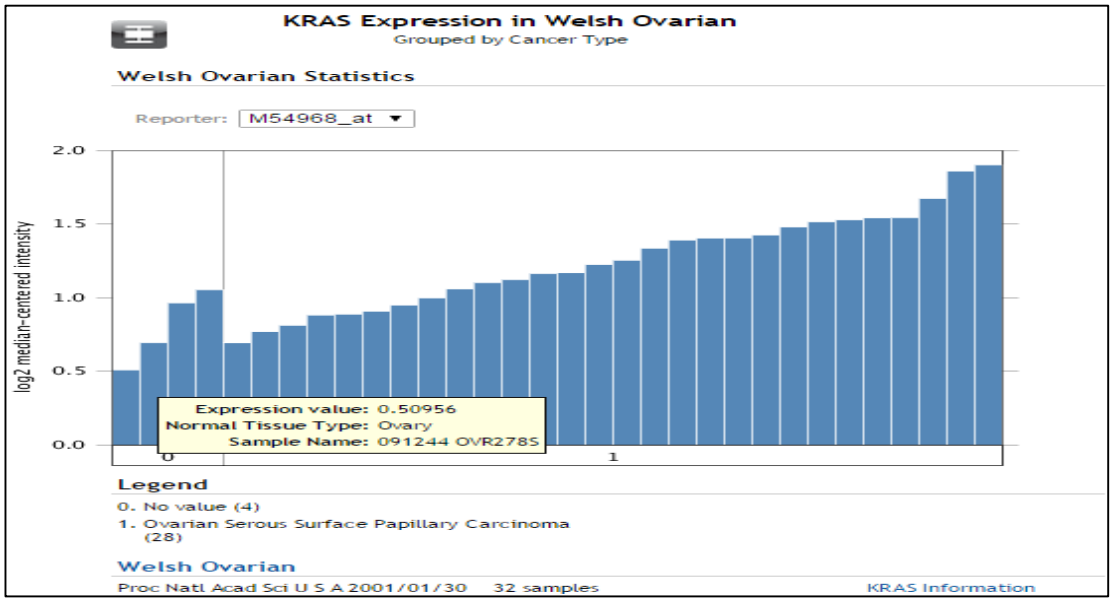

Figure-11. Elevated expression of kRAS in ovarian cancer

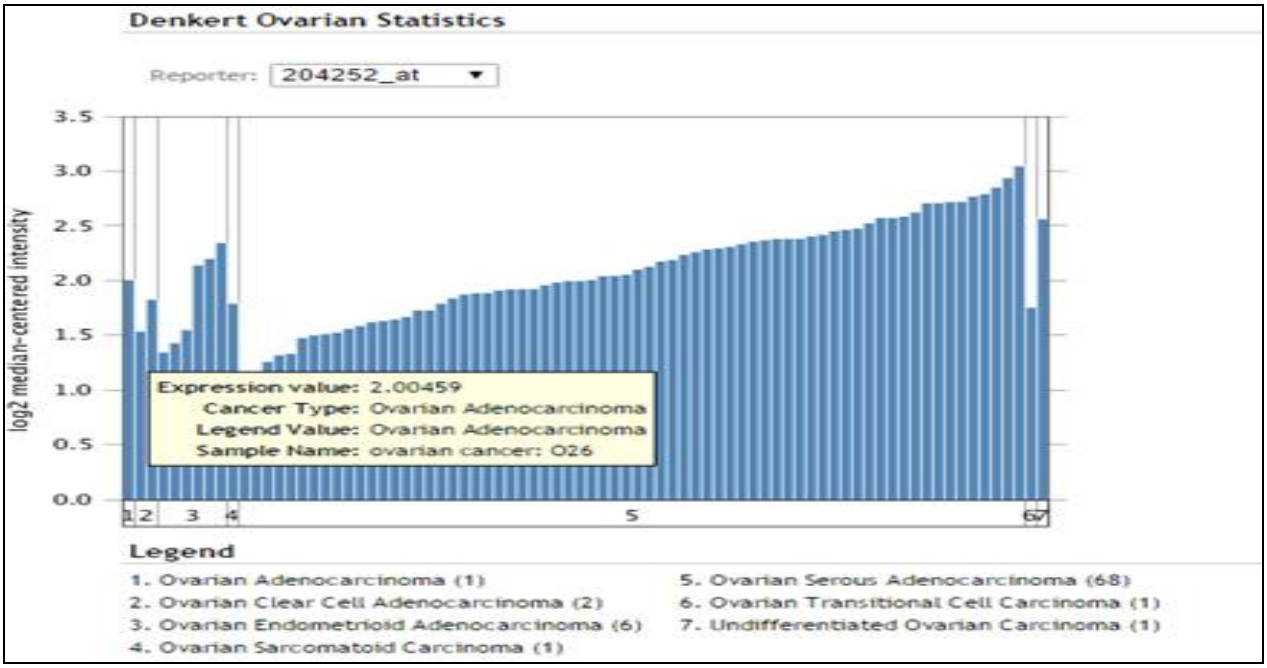

Figure-12. Elevated expression of CDK2 in ovarian cancer

6. Functional implication Putative miRSNPs

(a) rs61764370_kRAS- has-miR-223-5p

This miRSNP obtained was with destruction of binding site due to presence of SNP in the 3ðUTR of kRAS. Thus with destruction of existing site in the mutated allele, the miRNA is unable to target and the target transcript is now free from the RNA silencing machinery of the miRNA thus having elevated expression in OC. The expected expression of the transcripts was co-related with that of our Oncomine result.

Kirsten rat sarcoma (KRAS) viral oncogene homolog belongs to a class of known oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous. The *KRAS* gene is in the Ras family of oncogenes. These proteins play important roles in cell division, cell differentiation, and the self-destruction of cells (apoptosis).Some gene mutations are acquired during lifetime and are present only in certain cells. These changes, which are called somatic mutations, are not inherited. Somatic mutations in the *KRAS* gene are involved in the development of several types of cancer. The KRAS-variant is an inherited, germline variant that

has been demonstrated to serve as a genetic marker of increased risk of OC (Keane and Ratner, 2010).

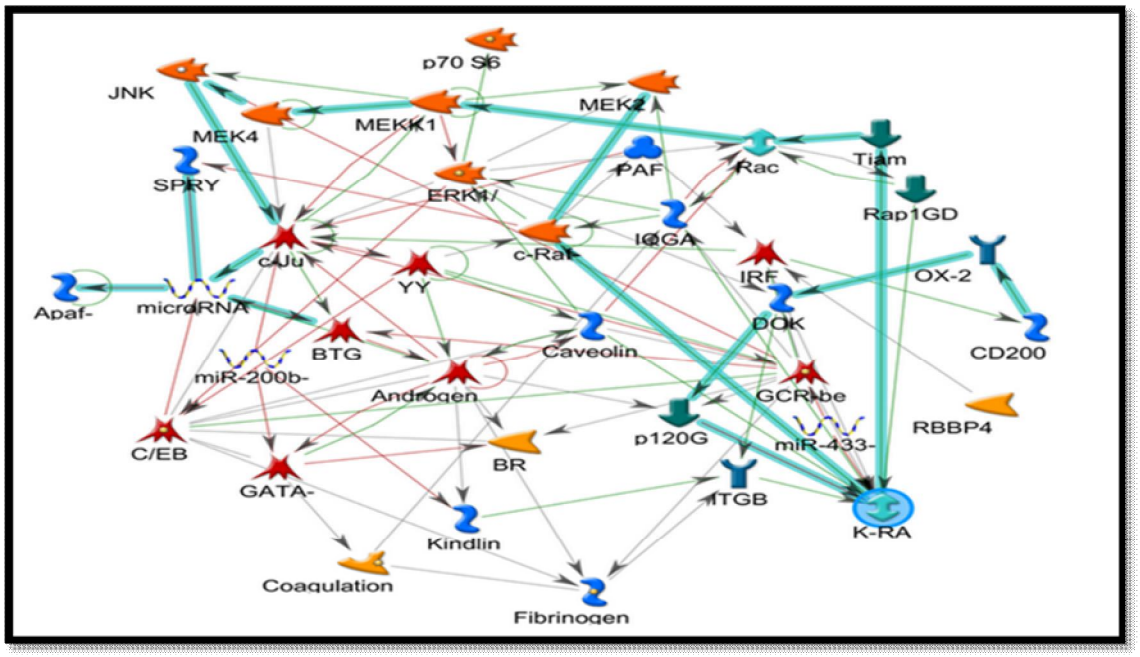

Figure-13. Networks showing KRAS & regulating downstream partners in OC

Gene	Object Type	Patners	Partner Type	Effect	Mechanism
K-RAS	RAS - superfamily	Tiam1	Regulators (GDI, GAP, GEF)	Activation	Binding
K-RAS	RAS - superfamily	c-Raf-1	Protein kinase	Activation	Binding

Table 3 CDK2 regulating other downstream interacting partners

(b) rs2069414_CDK2-has-miR-183-5p

The RNA Hybrid result for this miRSNP showed the destruction of binding site for this miRNA thus making the transcript CDK2 free from RNA silencing mechanism. Thus we hypothesize the over expression of CDK2 as shown in the Oncomine output is may be due the SNP causing the destruction target site for the miRNA.

Cyclin dependent kinases (Cdks), a family of serine kinases, faithfully control the mammalian cell cycle by binding to cyclins. Cdks play a key role in controlling the cell cycle, a process whose dysregulation can potentially lead to uncontrolled cell growth and leads to cancer. Cyclin E binds G1 phase Cdk2, which is required for the transition from G1 to S phase while binding with Cyclin A is required to progress through the S phase. Its activity is also regulated by phosphorylation. In normal cell CDK2is inhibited after one cell cycle while case of cancer cell the action of CDK2 is always high.

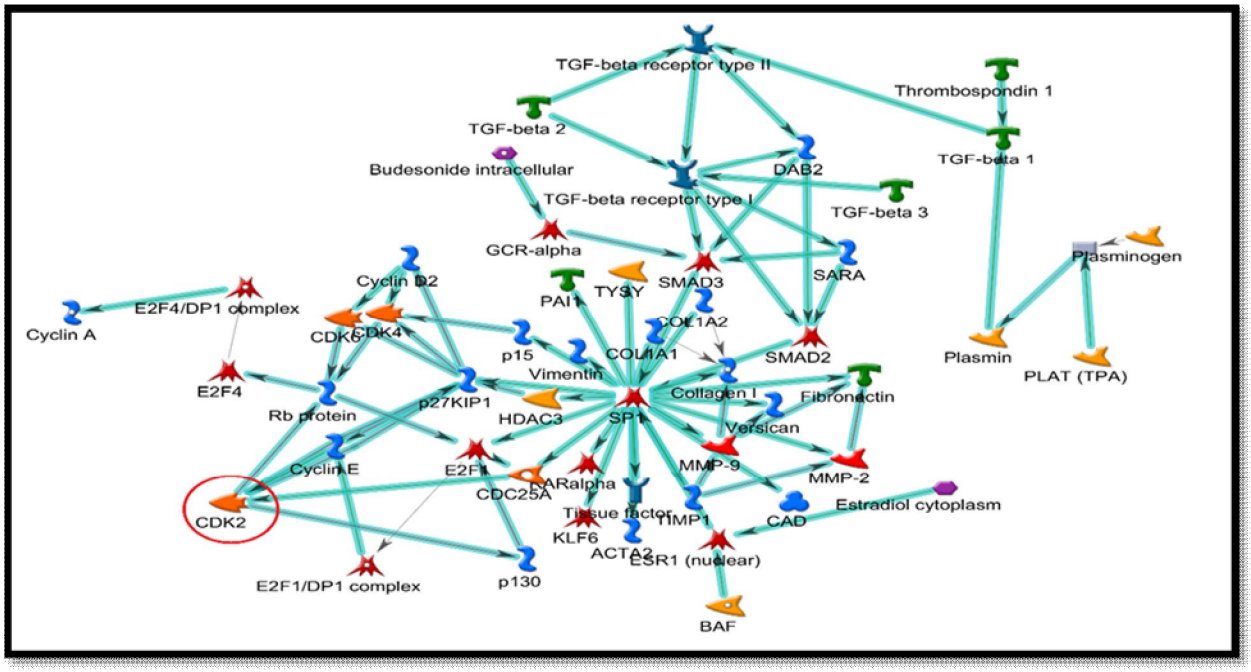

Figure-14. Networks showing CDK2 regulating downstream partners in OC

Gene	Object Type	Patners	Partner Type	Effect	Mechanism
CDK2	Protein kinase	PR (nuclear)	Transcription factor	Activation	Phosphorylation
CDK2	Protein kinase	p130	Generic binding protein	Inhibition	Binding
CDK2	Protein kinase	NEK1	Protein kinase	Unspecified	Phosphorylation
CDK2	Protein kinase	SMAD3	Transcription factor	Inhibition	Phosphorylation
CDK2	Protein kinase	Lyn	Protein kinase	Unspecified	Phosphorylation
CDK2	Protein kinase	SKP2	Generic binding protein	Inhibition	Phosphorylation
CDK2	Protein kinase	p21	Generic binding protein	Inhibition	Phosphorylation
CDK2	Protein kinase	FKHR	Protein	Inhibition	Phosphorylation
CDK2	Protein kinase	p27KIP1	Generic binding protein	Inhibition	Phosphorylation
CDK2	Protein kinase	E2F1	Transcription factor	Inhibition	Phosphorylation
CDK2	Protein kinase	Rb protein	Generic binding protein	Inhibition	Phosphorylation
CDK2	Protein kinase	ESR1 (nuclear)	Transcription factor	Activation	Phosphorylation
CDK2	Protein kinase	Cyclin E	Generic binding protein	Inhibition	Phosphorylation

Table-4. CDK2 regulating other downstream interacting partners

The targetscan analysis employed for establishing one gene-one miRNA hypothesis showed the possibility of another up regulated miRNA (has- miR-155) from our set with conserved binding site for the KRAS gene. But our further analysis showed this miRNA has another probable target i.e. DNMT3A (DNA Methyl transferase 3A) which was also found to have low expression in OC as resulted from Oncomine. Thus we hypothesize the low expression of DNMT3A may be due to has-miR-155 binding , which indicated that DNMT3A may be acting as a ceRNA (competitive endogenous RNA) against KRAS competition for the same miRNA.

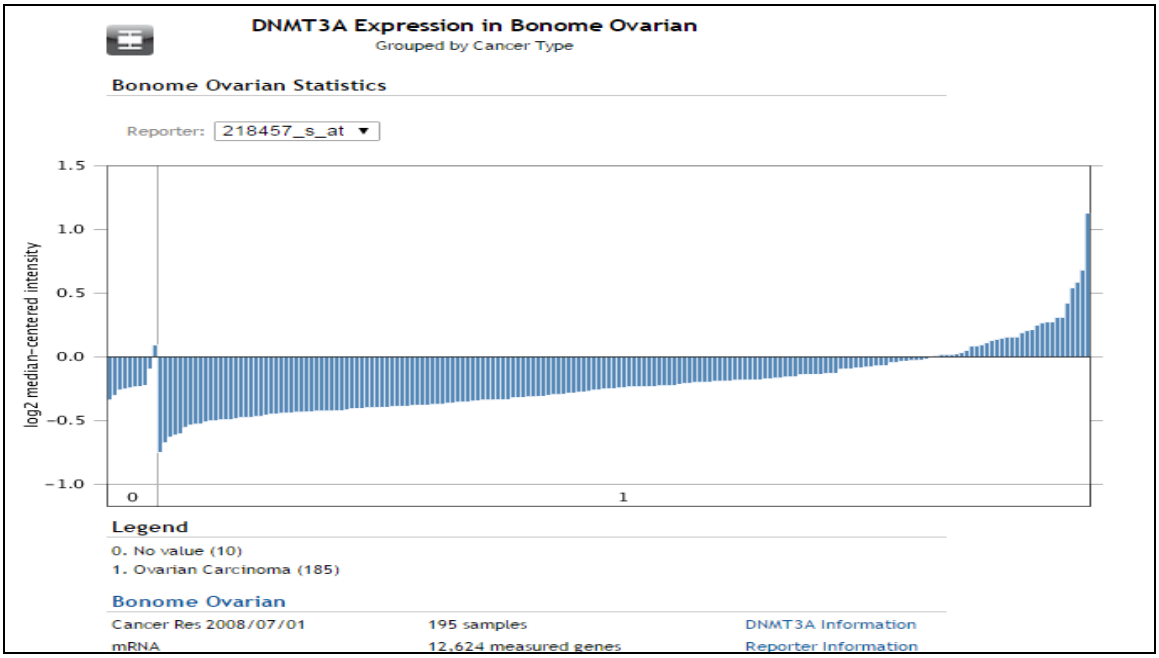

Figure-15. Low expression of DNMT3A in OC

Further analysis of the miRSNP pair in order to find out other binding site for the same miRNA present in the pair using RNA Hybrid (BB server) showed additional single binding site in the 3ðUTr of the respective genes both for KRAS and CDK2. This result indicates loss of additive effect of the in the UTR because of destruction of previously existing site due to SNP thus making the miRNA less effective in targeting the corresponding mRNAs.

```
Version: RNAhybrid 2.2
Command line:/vol/bioapps/bin/RNAhybrid.bin -e -10 -n 22 -m 5128
08_191919_5JVIt/rnahybrid_input_mirna_sequences.file -f 2,7 -t /v
searching
dataset: 1
mde of hsa-miR-223-5p: -42.700001
Individual hits
-----------------------------------------------------------------

dataset: 1
target: gi|575403058:762-5889
length: 5128
miRNA : hsa-miR-223-5p
length: 22

mfe: -18.2 kcal/mol
p-value: 1.000000e+00

position  1190
target 5'     C     G                U 3'
               CAGU UUGUC   AUGCAU
               GUCG AACAG   UAUGUG
miRNA  3' UUGA           UU       C 5'

-----------------------------------------------------------------
```

Figure-16. RNA hybrid showing additional binding site for KRAS-miR-223-5p pair

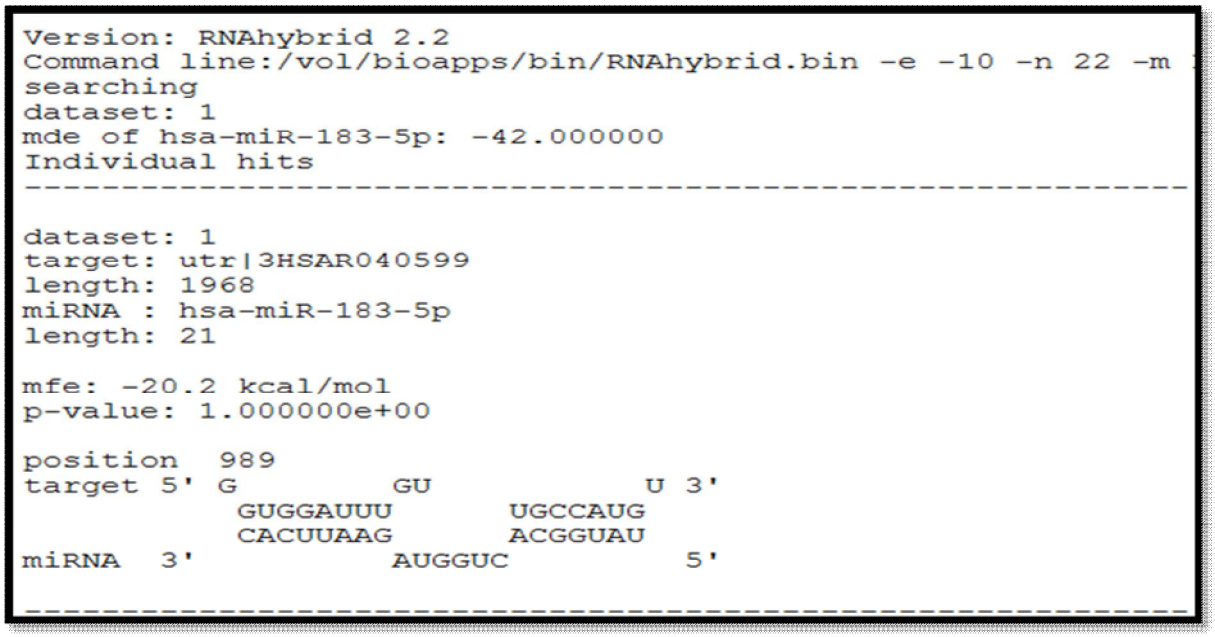

Figure-17. RNA hybrid showing additional binding site for CDK2-miR-183-5p pair

CONCLUSIONS

Conclusions

Our present study resulted in identification of two putative miRSNPs having functional significance in ovarian tumorigeneseis. From 137 pair obtained from the RNA Hybrid, 46 miRSNPs were filtered on the basis of different parameters like- change of binding site, variation in Seed topology, MFE change, presence GU in the seed site and Presence of additional binding sites beyond seed site. In rs61764370_kRAS-hsa-miR-223-5p miRSNP obtained, destruction of binding site was observed due to presence of SNP in the 3ðUTR of KRAS. Thus with destruction of existing site in the mutated allele, the miRNA is unable to target making KRAS free from the RNA silencing machinery of the miRNA thus having elevated expression in OC. Similarly RNA Hybrid result for miRSNP rs2069414_CDK2-hsa-miR-183-5p showed the destruction of binding site for this miRNA. Thus we hypothesize the over expression of CDK2 as well as KRAS may be due the SNP causing the destruction target site for respective miRNA. The functional significance of the pair was obtained from the GO ontology analysis by Metacore showed the regulatory aspect of KRAS and CDK2 interacting with other downstream molecule thus making the two genes to act as a hub in the biological network.

In future work the target validation of the miRSNPs can be done through luciferase assay. In addition to that, by silencing this genetically variable genes contributing to the cancer progression can be implemented in therapeutic prevention of this disease.

REFERENCES

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. Cell *136*, 215-233.

Brodersen, P., and Voinnet, O. (2009). Revisiting the principles of microRNA target recognition and mode of action. Nat Rev Mol Cell Biol *10*, 141-148.

Cargill, M., Altshuler, D., Ireland, J., Sklar, P., Ardlie, K., Patil, N., Shaw, N., Lane, C.R., Lim, E.P., Kalyanaraman, N.*, et al.* (1999). Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet *22*, 231-238.

Carvajal-Carmona, L.G., O'Mara, T.A., Painter, J.N., Lose, F.A., Dennis, J., Michailidou, K., Tyrer, J.P., Ahmed, S., Ferguson, K., Healey, C.S.*, et al.* (2015). Candidate locus analysis of the TERT-CLPTM1L cancer risk region on chromosome 5p15 identifies multiple independent variants associated with endometrial cancer risk. Hum Genet *134*, 231-245.

Charbonneau, B., Moysich, K.B., Kalli, K.R., Oberg, A.L., Vierkant, R.A., Fogarty, Z.C., Block, M.S., Maurer, M.J., Goergen, K.M., Fridley, B.L.*, et al.* (2014). Large-scale evaluation of common variation in regulatory T cell-related genes and ovarian cancer outcome. Cancer Immunol Res *2*, 332-340.

Erichsen, H.C., and Chanock, S.J. (2004). SNPs in cancer research and treatment. Br J Cancer *90*, 747-751.

Friedman, R.C., Farh, K.K., Burge, C.B., and Bartel, D.P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. Genome Res *19*, 92-105.

Gau, D.M., Lesnock, J.L., Hood, B.L., Bhargava, R., Sun, M., Darcy, K., Luthra, S., Chandran, U., Conrads, T.P., Edwards, R.P.*, et al.* (2015). BRCA1 deficiency in ovarian cancer is associated with alteration in expression of several key regulators of cell motility - A proteomics study. Cell Cycle, 0.

Han, J., Lee, Y., Yeom, K.H., Kim, Y.K., Jin, H., and Kim, V.N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev *18*, 3016-3027.

Holschneider, C.H., and Berek, J.S. (2000). Ovarian cancer: epidemiology, biology, and prognostic factors. Semin Surg Oncol *19*, 3-10.

Hwang, H.W., and Mendell, J.T. (2007). MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer *96 Suppl*, R40-44.

Kaku, T., Ogawa, S., Kawano, Y., Ohishi, Y., Kobayashi, H., Hirakawa, T., and Nakano, H. (2003). Histological classification of ovarian cancer. Med Electron Microsc *36*, 9-17.

Klein, C.A. (2008). Cancer. The metastasis cascade. Science *321*, 1785-1787.

Lancaster, J.M., Powell, C.B., Chen, L.M., and Richardson, D.L. (2015). Society of Gynecologic Oncology statement on risk assessment for inherited gynecologic cancer predispositions. Gynecol Oncol *136*, 3-7.

Lewis, B.P., Burge, C.B., and Bartel, D.P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell *120*, 15-20.

Mallick, B., and Ghosh, Z. (2011). A complex crosstalk between polymorphic microRNA target sites and AD prognosis. RNA Biol *8*, 665-673.

Meng, Q.H., Xu, E., Hildebrandt, M.A., Liang, D., Lu, K., Ye, Y., Wagar, E.A., and Wu, X. (2014). Genetic variants in the fibroblast growth factor pathway as potential markers of ovarian cancer risk, therapeutic response, and clinical outcome. Clin Chem *60*, 222-232.

Morlando, M., Ballarino, M., Gromak, N., Pagano, F., Bozzoni, I., and Proudfoot, N.J. (2008). Primary microRNA transcripts are processed co-transcriptionally. Nat Struct Mol Biol *15*, 902-909.
Murchison, E.P., and Hannon, G.J. (2004). miRNAs on the move: miRNA biogenesis and the RNAi machinery. Curr Opin Cell Biol *16*, 223-229.
Ramensky, V., Bork, P., and Sunyaev, S. (2002). Human non-synonymous SNPs: server and survey. Nucleic Acids Res *30*, 3894-3900.
Saunders, M.A., Liang, H., and Li, W.H. (2007). Human polymorphism at microRNAs and microRNA target sites. Proc Natl Acad Sci U S A *104*, 3300-3305.
Shastry, B.S. (2002). SNP alleles in human disease and evolution. J Hum Genet *47*, 561-566.
Shastry, B.S. (2009). SNPs: impact on gene function and phenotype. Methods Mol Biol *578*, 3-22.
Vignal, A., Milan, D., SanCristobal, M., and Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. Genet Sel Evol *34*, 275-305.
Wang, D., Lu, M., Miao, J., Li, T., Wang, E., and Cui, Q. (2009). Cepred: predicting the co-expression patterns of the human intronic microRNAs with their host genes. PLoS One *4*, e4421.
Zhou, L., Zhang, G., Zhou, X., and Li, J. (2015). The association between the SNP rs763110 and the risk of gynecological cancer: a meta-analysis. Biomed Pharmacother *69*, 208-213.
Kinose, Y., Sawada, K., Nakamura, K., and Kimura, T. (2014). The role of microRNAs in ovarian cancer. Biomed Res Int *2014*, 249393.
Wu, C., Gong, Y., Sun, A., Zhang, Y., Zhang, C., Zhang, W., Zhao, G., Zou, Y., and Ge, J. (2013). The human MTHFR rs4846049 polymorphism increases coronary heart disease risk through modifying miRNA binding. Nutr Metab Cardiovasc Dis *23*, 693-698.
Friedman, R.C., Farh, K.K., Burge, C.B., and Bartel, D.P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. Genome Res *19*, 92-105.
Lewis, B.P., Burge, C.B., and Bartel, D.P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell *120*, 15-20.